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1955 ANNUAL REPORT

of

VELVET MESQUITE INVESTIGATIONS

conducted by

WEED INVESTIGATIONS SECTION
FIELD CROPS RESEARCH BRANCH
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE

in cooperation with

UNITED STATES FOREST SERVICE
ROCKY MOUNTAIN FOREST AND RANGE EXPERIMENT STATION
Tucson, Arizona

by

Herbert M. Hull, Plant Physiologist
Weed Investigations Section

January 1956

SUMMARY OF PROGRESS

FC g1-1

Growth studies on velvet mesquite in temperature controlled greenhouses have shown optimum growth at a day temperature of 30° C. (86° F.) and a night temperature of 26° C. (79° F.). Ability to grow at these high temperatures and to survive under extreme ranges of soil moisture are factors which make possible aggressive invasion by this noxious plant.

Although herbicidal response of honey mesquite and velvet mesquite grown in the same greenhouse was not significantly different, growth rate of the honey variety was considerably the slower of the two during winter. With the advent of longer spring days growth accelerated in the honey mesquite, approaching that of velvet. The more rapid growth of the latter plant during mid-winter, in a heated greenhouse, suggests the possibility of different critical photoperiods (for growth) in the two varieties.

Mesquite seedling studies in which a wide range of oil to water ratios was used as a carrier for 2,4,5-T have demonstrated optimum kill with a water only carrier. Although similar results have been shown in hand spraying of field mesquite, an oil-water emulsion generally is better for aerial application. Other laboratory experiments have shown that by acidification of the carrier with trichloroacetic acid and emulsification with proper type and concentration of wetting agent, an increased absorption of 2,4,5-T may be obtained.

FC g1-2

A field study involving 20 combinations of carriers, 2,4,5-T formulations and emulsifier concentrations has demonstrated optimum kill with a 2,4,5-T ester in a diesel oil-water emulsion, with the addition of a high (1.2 percent) concentration of emulsifying agent.

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PUBLICATIONS

Carbohydrate relations in naturally-growing velvet mesquite. Research Progress Report, Research Section, Western Weed Control Conference, Boise, Idaho, March 22 - 23, 1955. Pp. 77-78.

INTRODUCTION

Physiological research at the Santa Rita Research Center has been concentrated upon velvet mesquite (*Prosopis juliflora* var. *velutina*). Although a number of other noxious range plants exist and are spreading on Southwestern ranges, the fact that mesquite now covers over 70 million acres makes it desirable that research on this plant be continued. There is always some possibility that findings relating to mesquite may be applicable to certain other noxious range plants.

Although mesquite can be killed satisfactorily on an individual tree basis, the extensive acreage of most ranches together with the relatively high density of mesquite growth in many cases, makes aerial spraying the only economically feasible method of control on most of the ranges. If the present average of 20 to 25 percent kill could be significantly raised, a greatly increased number of land owners would invest in aerial control of mesquite, and the infested range lands would benefit accordingly. It is toward this goal that physiological research on mesquite is currently directed.

ACKNOWLEDGEMENT

For the many suggestions and assistance given by the Forest Service personnel of the Rocky Mountain Forest and Range Experiment Station at Tucson, and also by faculty members of the University of Arizona, the author is deeply indebted.

On behalf of the Weed Investigations Section, appreciation is extended to Drs. G. W. Beadle and F. W. Went of the California Institute of Technology for the free offer of space in the greenhouses of the Earhart Plant Research Laboratory. Particular thanks are due Dr. Jorge Morello of Argentina who planted the mesquite seedlings and gathered much of the data reported in the growth experiment on page 5.

The assistance of various chemical companies for the gratis supply of products is greatfully acknowledged. Those whose products were utilized during 1955 include: Alrose Chemical Company, American Chemical Paint Company, Antara Chemicals, Colloidal Products Corporation, Dow Chemical Company, Heyden Chemical Corporation and Socony-Vacuum Oil Company.

GENERAL RESUME AND OUTLOOK

Research on the physiological aspects of mesquite and its control has progressed satisfactorily during 1955. Experiments have been carried out both in the laboratory and field. Although the homogeneity of greenhouse-grown plants gives many advantages, there are certain responses that can be properly evaluated only in the field.

Several cooperative projects are contemplated with the Department of Agronomy and Range Management of the University of Arizona. One, currently under review, deals with the annual variation of herbicidal sensitivity of burroweed (Haplopappus tenuisectus) and mesquite (Prosopis juliflora var. velutina) to 2,4,5-T, and the correlation of this sensitivity with carbohydrate and indole-3-acetic acid content. It will involve weekly or bi-weekly sampling over a one-year period. A second cooperative project deals with the radial growth increment of mesquite at four cardinal points on the lower trunk, and possible correlation of radial growth with carbohydrate content of the phloem and xylem of the taproot directly below these points. This study will also reveal variation in carbohydrate content on four sides of a given tree.

Research at Tucson during the year was carried out with the assistance of two graduate students from the University of Arizona, working on a two-day-per-week basis. One student was employed the first five months of the year, and the other for two months in the fall.

During the late fall and winter preliminary work was carried out on the development of microtechnique for mesquite examination. Plans for the immediate future call for a histological study of possible phloem inactivation by 2,4,5-T, and the factors influencing such inactivation. The necessary equipment for this study has now largely been procured.

Screening tests of promising herbicides will be continued during 1956. It is also hoped that a study plan dealing with the effect of certain mineral deficiencies upon herbicidal response of mesquite can be initiated when time permits.

In all experiments described in this report, herbicidal concentrations are given as their acid equivalents, and distilled water was used throughout. (Herbicides used for field application were made up with water from the Santa Rita Experimental Range, which has a total salt concentration of only 50 ppm.).

SUMMARY OF LINE PROJECT INVESTIGATIONS

FC gl-1 - DETERMINATION OF THE PHYSIOLOGICAL MECHANISMS INVOLVED IN THE SELECTIVE KILLING ACTION OF HERBICIDES

The relationship between herbicidal sensitivity and carbohydrate fluctuations in the root xylem of velvet mesquite.

The dependency of herbicidal translocation upon simultaneous movement of carbohydrates, either native or externally applied, has been demonstrated by several investigators. In a preliminary survey involving the annual variation of specific sugars and starch in various organs of the mesquite tree, rapid changes were noted during the spring months - the approximate period when the tree is considered to be most susceptible to herbicides. This finding suggested the installation of an experiment in which the trees would be sampled for carbohydrates and sprayed on the same day at frequent intervals during the susceptible period. Thus, samples of root xylem, the 3 mm. layer adjacent to the cambium, were taken at weekly intervals from April 29th to July 1, 1955, (semi-weekly during the mid-portion of this period). On each collection date two groups of trees were sprayed - one with 12,000 ppm. acid equivalent of an amine of 2,4,5-T carried in a 1:4 emulsion of nontoxic oil in water, and the other with a similar concentration of the PGBE ester of 2,4,5-T in a diesel oil-water emulsion. In case the period of maximum susceptibility is dependent upon herbicide formulation and type of carrier, the use of two separate treatments may help reveal possible differences.

Although starch content remained relatively stable throughout

the entire period, total sugars constantly increased from a value of 11.5 mg. per gram dry weight on April 29th to 38.6 on June 16. Maximum defoliation, read in October of the same year, occurred on plants treated during late May, approximately the mid-point of the sugar build-up period. Moisture content of the xylem remained relatively stable over the entire period, averaging about 31 percent of the dry weight. Soil temperature at 25 cm. depth (9:00 A.M.) ranged from a low of 67° F. on April 29th to a high of 90° on June 24th. Neither of these observations appear to have a direct correlation with defoliation, but may have some correlation with plant kill which will be read during the summers of 1956 and 1957.

The influence of day and night temperature upon the growth of velvet mesquite.

The generous offer of space in the air conditioned greenhouses at the California Institute of Technology made possible the study of mesquite growth under six different combinations of day and night temperature. Studies of this nature are valuable in determining optimum natural environmental conditions under which mesquite may be expected to reproduce and grow if allowed to spread unchecked.

Plants were germinated and grown at a day temperature of 30° C. with corresponding nights of 26°, 23° and 20°, and at a day temperature of 26° with nights of 23°, 20° and 17°. All plants received a 16 hour photoperiod consisting of eight hours natural light followed by eight hours artificial light of 2,000 footcandle intensity. The latter consisted of combined incandescent and fluorescent illumination, and had a spectral distribution similar to daylight.

Mean height and leaf counts were taken on the 33rd, 54th, 74th and 86th day after germination, for all groups. The plants were harvested at an age of 97 days, at which time fresh and dry weight of shoots and roots was tabulated. The actual shoot and root growth as determined by either weight, height, or leaf count, was greatest at a day temperature of 30° C. and a night temperature of 26°C. Mesquite is one of the first plants of the many which have been grown in the greenhouses at Pasadena that has exhibited optimum growth at the highest temperatures available. Its growth at a wide range of temperatures, together with its previously determined ability to germinate and grow at extreme ranges of soil moisture and aeration, demonstrate how such aggressive invasion is possible under conditions that are far from ideal for most plants.

One fresh plant from each temperature group was preserved for anatomical studies, and the dried plants are being preserved for studies on lipids and waxes in cooperation with the University of Arizona.

Preliminary studies on mineral nutrition and the influence of nutrition on herbicidal response.

In order to avoid osmotic effects and antagonistic effects among ions, it is desirable to carry out physiological investigations at the minimum nutrient concentration consistent with optimum growth. Since earlier experiments had demonstrated that velvet mesquite grew slightly better in 50, 25 and even 10 percent Hoagland's nutrient than in 100 percent, an experiment was set up utilizing 10, 5 and 2 percent nutrient concentrations. The solution was renewed bi-weekly and adjusted to pH 6.0 at each renewal, the optimum for mesquite. Plants cultured under

the three conditions all grew to equal heights, and only those at the lowest level were slightly chlorotic, in spite of added iron.

To test the tolerance of mesquite for soils of high salinity, where it is often found growing, plants were grown in 25 percent Hoagland's nutrient at four levels of sodium chloride, namely 0, 500, 2,000 and 10,000 ppm. Strangely enough, plants grown at the 2,000 ppm. level, although variable, were slightly larger than those grown at the two lower levels. (Two-month-old plants had a mean height of 17.4 cm. compared with 14.0 and 13.3 cm.) The highest salt concentration was lethal to the majority of the plants. The experiment demonstrates the ability of mesquite to grow at relatively high salt concentrations, and suggests the possibility of a small sodium requirement. Further work would need to be carried out, however, to establish such a requirement.

Another element that is highly variable and often deficient in Southwestern soils is phosphorous (P). In order to establish whether mesquite growing on a soil deficient in this element has a different herbicidal sensitivity from trees growing on a non-deficient soil, an experiment was designed in which 16 plants were grown at each of four levels of P in culture solutions (Hoagland's) of 25 percent strength. P concentrations of 0, 0.08, 0.8 and 8 ppm. were used. At an age of two months, 5,000 ppm. of the triethylamine salt of 2,4,5-T in a 1:4 emulsion of nontoxic oil in water was applied to the central five leaves of 10 plants in each group. Untreated plants were maintained in the deficient nutrients, and the mean height of these four groups at an age of three months was 9.7, 38.0, 45.5 and 64.6 respectively, for the four levels of P. Spectrophotometric determination of total P

was made on the shoots and roots of non-treated plants that had been growing in the four nutrients of varying P content. In the shoots respective values of 3.9, 4.3, 6.8 and 14.2 mg. per gram dry weight were found, whereas corresponding values in the roots were 1.5, 1.8, 3.9 and 6.4.

As to the treated plants, initial herbicidal responses were not significantly different among any of the four groups. After the third week, however, the entire apical portions of the plants lacking P were dead, and percentage of kill after three months was 60, 20, 0 and 10 respectively for the four P levels. None of the non-treated plants died, even at zero P.

Comparative growth and herbicidal response of honey mesquite and velvet mesquite.

Whether or not the honey mesquite (Prosopis juliflora var. glandulosa) of Texas, Oklahoma and New Mexico and the velvet mesquite (P. juliflora var. velutina) of Arizona respond similarily to herbicides is a question that has often been asked. It would be desirable to know whether certain responsive differences are due to environmental differences common to the individual ranges or are due to actual differences between the two plants. In order to help clarify the above question, 30 plants of each variety were grown from seed in the greenhouse at Tucson. Although germination percentage was greater in the honey mesquite than in the velvet, the latter grew considerably more rapidly. Respective mean heights at an age of 3 months were 4.0 and 22.2 cm., the individual groups being very homogeneous. In order to give equally effective herbicidal treatments, the central leaves of

each plant, which amounted to approximately one-third of the total foliage, were painted with the triethylamine salt of 2,4,5-T, 5,000 ppm. Overall responsive differences between the groups were not great. Although greater form tive effect and injury was evident in the apical portions of the honey mesquite, the basal non-treated leaves of the velvet variety showed more chlorosis. Whereas the stem dieback was greater in the velvet mesquite, the only plant to die of the total of 20 treated was a honey mesquite.

Growth rate of the non-treated honey mesquite plants began to pick up with the advent of longer spring days, and approached that of velvet mesquite. It seems probable that honey mesquite may have a critical minimum photoperiod for growth, and that the day length in mid-winter is shorter than the critical period. Temperature as a limiting factor of growth in this experiment was unlikely, as the greenhouse was heated during the winter.

As to overall herbicidal responses of the two varieties, differences were not of a sufficient magnitude to form the conclusion that one variety is more readily killed than the other.

Effect of the ratio of oil to water in herbicidal carriers.

Although experiments involving aerial application of 2,4,5-T generally (though not always) indicate an oil-water emulsion to be superior to water only as a carrier, ground application is usually most effective with a water carrier. In addition, greenhouse experiments have shown optimum results with a water carrier. To establish exactly what ratio of oil to water in the carrier is the most effective for an

amine of 2,4,5-T, an experiment was set up utilizing the following ratios of nontoxic oil to water (by volume): 100 percent oil, 1:1, 1:4, 1:9, 1:29 and 100 percent water. The triethylamine salt of 2,4,5-T at 5,000 ppm. was used in all carriers containing water, and an oil-soluble amine of 2,4,5-T at an equivalent acid concentration was used in the straight oil carrier. An anionic emulsifier (0.3 percent) was added to all mixtures.

Application to the central leaves of all plants resulted in spotted bleaching by the fifth day. Severity of bleaching was moderate in five of the treatments, but was significantly lighter in the case of the water-only carrier. Formative effect and chlorosis to the apical portion of the plant and to the non-treated basal leaves was approximately equivalent with all treatments. However, two months after treatment, there were significant differences in stem dieback as a percentage of total stem length. The mean values, from straight oil to straight water carrier, were respectively: 20, 45, 48, 33, 46 and 68. In addition, of the four plants which ultimately died out of a total of 60, all were from the group treated with the water-only carrier. The data from this experiment, along with that of numerous field experiments, suggest that the use of water as a carrier should not be completely discontinued in future studies.

The effect of pH upon herbicidal efficiency of the free acid of 2,4,5-T.

Several experiments were run utilizing granules of the free acid of 2,4,5-T in a 1:4 emulsion of nontoxic oil in water, adjusted to various pH levels. In the initial experiment the herbicide mixtures

were adjusted to pH values of 2.0, 4.0, 6.0 and 8.0 with trichloroacetic acid (TCA) and ammonium hydroxide. Forty plants at the three-leaf-stage were selected, and the herbicide was painted on the upper surface of the cotyledons only. Contact injury was not directly correlated with pH, but was least at the two extreme values of 2.0 and 8.0. All post-treatment responses including initial apical epinasty, chlorosis of the apical portion of the stem and young leaves, dieback of the stem, and eventual percentage kill (20 percent) were greatest in the pH 2.0 group.

In a second experiment, when the same herbicide of varying pH was applied to centrally located leaves rather than cotyledons, resultant contact injury was still least at the lowest pH of 2.3, but the difference between pH levels was considerably less than when the cotyledons were treated. Although stem callus was most profuse in the pH 2.3 group, formative effects and chlorosis to the apical portion of the plants were most severe at pH 4.0. It is conceivable that actual absorption was greatest at pH 2.0, and that upon reaching the stem adjacent to nodes of the treated leaves in a relatively large quantity, the herbicide inactivated the phloem (as evidenced by the callus formation at pH 2.0) sufficiently to inhibit herbicidal translocation beyond the calloused region. Histological studies of the phloem are contemplated which should help clarify whether or not such responses represent a true inactivation. Additional work incorporating various pH levels in conjunction with different herbicidal concentrations would be desirable to help elucidate the problem.

A further experiment utilized 90 plants at the three-leaf stage, divided into two equal groups. On one group the cotyledons were treated

with the above mentioned herbicide adjusted to each of three pH levels (2.0, 5.0 and 8.0). Each plant of the second group had the primary leaf so treated. To avoid possible superimposed herbicidal effects resulting from the TCA, pH values were adjusted with phosphoric acid. The lessened contact injury to the cotyledons at pH 2.0 did not follow in this case, as when acidity was adjusted with TCA. Initial apical epinasty was greater in the leaf-treated group as a whole, and within this group was greatest at the pH 2.0 and 5.0 levels. Later formative effects and chlorosis to the plant apices and young leaves were equivalent in the two major groups, but within both groups, maximum effects were obtained at pH 5.0. These experiments demonstrate that definite beneficial effects may be achieved by increasing the acidity of the herbicidal mixture, and suggest TCA as being the most likely additive to give optimum results.

The influence of emulsifier concentration upon herbicidal effectiveness on field grown velvet mesquite, accompanied by different forms of 2,4,5-T and different carriers.

Relative importance of emulsifier type and concentration in greenhouse evaluations (Annual Report, 1954) suggested the advisability of a field study involving this variable. The study was installed during September of 1954, and utilized two carriers, one a 1:4 emulsion of non-toxic oil in water, and the other a similar emulsion of diesel oil in water. Two individual formulations of 2,4,5-T (12,000 ppm.) were used with each carrier, namely the triethylamine salt and the butoxyethanol ester. Thus, four basic herbicidal mixtures were used, and each of these had an anionic emulsifier added at five different concentrations (0.0, 0.1, 0.4, 0.8 and 1.2 percent by volume), making a total of 20 treatments. Twenty trees in widely separated replicate plots of 10 each were hand sprayed with the herbicide mixtures, a total of 400 trees.

The initial observation was taken one month after treatment, and a second observation on the percentage of foliage remaining on trees was taken during September of 1955. The latter observation, which gives a fairly good prediction of kill, indicated optimum results with the 2,4,5-T ester-diesel oil emulsion combination. Only within this one basic treatment was a positive correlation between emulsifier concentration and herbicidal effectiveness obtained. At the high emulsifier concentration of 1.2 percent, a mean of only 16 percent of the foliage remained on the trees - far less than with any other treatment (range of other 19 treatment means was 28 to 65 percent). This fact suggests

that in future experiments an even higher concentration of emulsifier should be employed.

An analysis of variance, including the 20 treatments, indicated highly significant differences between carriers, emulsifier concentrations, and the interactions of carrier by herbicidal formulation and emulsifier concentration by formulation. Differences between formulation alone, and other combinations of the three variables were non-significant.

Absorption and translocation of several relatively new herbicides in velvet mesquite seedlings.

Screening tests of certain relatively new herbicides of the chlorophenoxy group were continued during 1955. In all cases the herbicide was used at 5,000 ppm. acid equivalent, and was carried in a 1:4 emulsion of nontoxic oil in water, with the addition of 0.2 or 0.3 percent of an anionic emulsifying agent. Application was always to the upper surface of the central leaves, which made up approximately one-third of the foliage. The previously described responses to apical and basal non-treated portions of the plant at various intervals after treatment are good indicators of absorption and/or translocation.

The following herbicides were evaluated in the above manner during 1955: 2,4,5-T, free acid granules containing 90 percent Bentonite filler; 2,4,5-T, triethylamine salt; 2,4,5-T, butoxyethanol (BE) ester (two formulations); 2(2,4,5-trichlorophenoxy) propionic acid, mixed ethanol and isoproponol amines (2,4,5-TP amine); 2(2,4,5-trichlorophenoxy) propionic acid, propylene and polypropyleneglycolbutylether esters (2,4,5-TP ester); 2(2,4-dichlorophenoxy) propionic acid, mixed ethanol and isoproponol amines (2,4-DP amine); 2(2,4-dichlorophenoxy)

propionic acid, mono, di and tripropyleneglycolbutylether esters (2,4-DP, PGBE ester) and 2(2,4-dichlorophenoxy) propionic acid, butoxyethanol ester (2,4-DP, BE ester).

Considering the overall responses, the free acid and the amine salt of 2,4,5-T gave considerably better results than any of the other herbicides, under the conditions of the experiments. Of the five propionic acids, the two amine formulations of 2,4-DP and 2,4,5-TP resulted in least contact injury but slightly better overall effect than the corresponding PGBE esters or the BE esters of 2,4-DP and 2,4,5-TP. On comparing the relative herbicidal effect of 2,4-DP with 2,4,5-TP (amine for amine or ester for ester), there are probably no significant differences.

Herbicidal effects of 2,4,5-T, amino triazole and trichlorobenzoic acid, alone and in various combinations.

Although amino triazole (AT) has shown marked physiological effects on velvet mesquite seedlings (Annual Report, 1954), it does not appear to have the killing power of the chlorophenoxy compounds for this plant. However, it was considered desirable to test for its possible synergistic effect when used as an additive to 2,4,5-T, as well as the effects of 2,3,6-trichlorobenzoic acid (TCB) and its sodium salt (NaTCB), alone and in various combinations with 2,4,5-T and AT. In this experiment, all herbicides were carried in a 1:4 emulsion of nontoxic oil in water, with the addition of 0.3 percent of an anionic emulsifying agent. Individual herbicides, alone or in combination, were used at 5,000 ppm. acid equivalent, except as noted. The 11 mixtures included: 2,4,5-T, TCB , NaTCB, NaTCB (10,000 ppm.), NaTCB (20,000 ppm.), 2,4,5-T + AT,

2,4,5-T + TCB , 2,4,5-T + NaTCB, AT + TCB , AT + NaTCB and TCB + NaTCB.

A total of 110 plants, 10 per group, were treated by painting the upper surfaces of the central four leaves. Translocation to and resultant injury of the non-treated portions of the plants, as evidenced by previously described responses, were considerably greater with 2,4,5-T alone than with any other herbicide or combination of herbicides. Although marked contact injury to treated leaves occurred with all 11 mixtures, the addition of AT to either 2,4,5-T or TCB significantly reduced contact injury resulting from the latter herbicides alone. The three concentrations of NaTCB gave approximately equivalent results.

An unusual response was the "micro leaves" which formed from sprouts resulting from treatment with TCB or NaTCB alone or in certain combination with other herbicides. Many such leaves, although seemingly physiologically mature, never reached a size greater than approximately one-fourth that of mature leaves.

FC gl-1 - DETERMINATION OF THE PHYSIOLOGICAL MECHANISMS INVOLVED IN THE SELECTIVE KILLING ACTION OF HERBICIDES

The relationship between herbicidal sensitivity and carbohydrate fluctuations in the root xylem of velvet mesquite.

The rather wide annual fluctuation in concentration of certain carbohydrates within the phloem and xylem of mesquite trunks and roots has been noted in preliminary work. To correlate possible relationship between herbicidal sensitivity on a number of given dates and carbohydrate content of the root xylem on the same dates, the present experiment was designed. It is described in detail in Research Work Plan No. 2, Line Project No. A-10-1-1 (FC-gl-1).

Essentially, the plan calls for spraying 10 moderate-sized vigorous trees with 12,000 ppm. of an amine of 2,4,5-T (S-1158, Dow) in a 1:4 emulsion of nontoxic oil (S/V Sovaspray 100, Socony-Vacuum) in water. In a similar manner, 10 trees were sprayed with the propyleneglycolbutylether ester of 2,4,5-T in a diesel oil-water emulsion. On each of 12 spraying dates throughout the spring and early summer growing season, during which mesquite is known to be most susceptible to foliage application of herbicides, samples of the taproot xylem were also taken, as described in the study plan.

In addition to total sugars and total starch, the moisture content of the tissues was determined and the soil temperature was taken at a depth of 25 cm. at 9:00 A.M. Minimum temperature the same morning and maximum temperature the day previous was also recorded. The above mentioned data is presented in table 1 together with mean defoliation resulting from the two treatments. No rain fell during the entire period of the experiment, nor during the five weeks prior to its initiation.

Table 1. Variation in temperature, root moisture and carbohydrate content during the period of frequent interval spraying, and relation to defoliation four to five months later.

Date of spraying, 1955	Air temperature, °F		Soil temperature, °F. at 25 cm. depth, 9 A.M.	Moisture content of root xylem 1/ (percent of dry wgt.)	Total sugar 1/ (mg./g. dry wgt.)	Total starch 1/ (mg./g. dry wgt.)	Percent defoliation 2/ on 10/14/55
	Minimum	Maximum on day previous					
4-29	54	90	67	32.0	11.5	113.8	60
5-6	58	92	73	32.1	13.2	112.9	62
5-13	59	93	74	31.0	13.8	99.0	85
5-20	55	81	70	31.0	19.5	114.2	81
5-26	49	75	72	32.6	26.8	100.8	93
5-30	68	101	76	30.6	23.5	97.9	92
6-3	50	84	74	30.6	31.4	117.1	76
6-7	65	98	77	30.8	32.1	98.6	72
6-10	76	109	82	30.7	29.5	95.2	70
6-16	64	97	82	30.1	38.6	124.8	76
6-24	76	109	90	36.1	34.7	129.1	40
7-1	66	96	83	28.7	33.8	115.1	84

1/ Mean of four samples which included three trees each.
2/ Mean of two treatments which included ten trees each.

Although sugar and starch did not reach a maximum until mid-June, defoliation was greatest on plants which were sprayed during the last week of May. Actually, accumulation of sugars in particular were fairly constant during the period from April 29th to mid-June, the daily average increment being about 0.56 mg. per gram of dry tissue. It seems evident that the most susceptible period did not fall at either the beginning or the end of this sugar build-up, but approximately at the middle of it. Starches remained fairly constant during most of the sampling period, and only built up slightly toward late June.

In making correlations with defoliation, it should be kept in mind that even late defoliation (read October 14, 1955) may not necessarily bear a relationship to final kill, which will be read during the summer of 1957 for this experiment. No particular correlation of either soil temperature or xylem moisture content with defoliation was noted. Values of tissue moisture remained remarkably constant, not only on given dates where variation among samples was generally not over 2 percent, but also from date to date as may be seen by the mean values in table 1. The slightly higher value on July 24th is likely real, but may be partially due to the fact that a different individual collected the samples on that one day.

Interpretation of the data from this experiment is complicated by the fact that carbohydrate reserves stored in the roots are utilized during active growth. Time of year of greatest root growth has not been investigated for mesquite. A knowledge of this factor would perhaps make the carbohydrate picture more intelligible. A further possibility that should be considered is that the amount of foliar applied herbicide

translocated into the roots is not necessarily only a function of the volume of basally moving carbohydrates, but is also a function of ease of penetration, degree of phloem inactivation and other factors. Future experiments should help resolve some of these individual factors.

The influence of day and night temperature upon the growth of velvet mesquite.

Relatively little work has been done on the ecological aspects of mesquite reproduction and growth. One factor of prime importance in determining the growth rate and the ultimate distribution of mesquite, if it is allowed to spread unchecked, is day and night temperature. The offer of space in the Earhart Plant Research Laboratory at the California Institute of Technology made the study of such temperature effects on growth possible.

Details of this study are outlined in Research Work Plan No. 3, Line Project No. FC-gl-1. In each of the 6 temperature combinations indicated in table 2, 16 plants were germinated and grown in plastic containers 15 cm. high by 9 x 9 cm. With the exception of air temperature, all temperature combinations received indentical treatment, and were subjected consecutively to eight hours of natural light, eight hours of artificial light and eight hours of darkness. The artificial light source consisted of daylight fluorescent tubes operated under boosted voltage in combination with incandescent lamps. The resultant illumination had a spectral distribution similar to daylight and an intensity of about 2000 footcandles at the plant apices.

Due to limited space in the greenhouse, the 12 most vigorous plants in each group of 16 were selected at an early age and growth measurements and leaf counts were taken on three representative plants in each group at regular intervals as indicated in table 2. At time of harvest (age, 97 days) one plant of each group was preserved in FAA killing solution for future anatomical study. Fresh and dry

Table 2. Shoot and root weight, height and number of leaves on velvet mesquite plants grown at various day and night temperatures.

Temperature, °C.		Mean weight per plant 1/ at age of 97 days (grams)				Mean height of plants 2/ at ages indicated (cm.)				Mean number of leaves on plants 2/ at ages indicated			
		Fresh weight		Dry weight									
Day	Night	Shoot	Root	Shoot	Root	33 days	54 days	74 days	86 days	33 days	54 days	74 days	86 days
30	26	11.76	1.83	3.88	0.47	33.3	63.5	123.5	164.5	17	36	57	68
30	23	6.36	1.23	2.05	0.29	28.2	53.3	87.0	109.0	15	25	37	44
30	20	4.02	0.87	1.27	0.19	21.2	42.6	69.1	85.0	9	20	30	35
26	23	10.90	1.66	3.40	0.42	29.1	65.1	111.5	145.0	14	29	44	51
26	20	2.81	0.67	0.93	0.17	19.2	42.0	57.0	67.0	9	18	23	27
26	17	1.49	0.47	0.49	0.10	13.1	26.1	45.5	52.5	6	13	19	22

1/ Mean of 11 plants.
2/ Mean of three representative plants.

weights of shoots and roots were determined on the others in three groups of three plants each and one group of two plants for each temperature condition.

The above data are shown in table 2. The percentage of dry to fresh weight was calculated for shoots and roots under all conditions. Values ranged only from 31.2 - 33.2 percent in the case of shoots, and appeared to have no particular relation to plant weight or temperature distribution. Values for roots ranged from 21.2 - 25.5 percent, with the higher values being at higher night temperatures.

Actual shoot and root growth as determined by either weight or height, or by leaf count, was greatest at a day temperature of 30° C. and a night temperature of 26° C. (figures 1, 2, and 3). These are the highest temperatures normally available in the greenhouse, and velvet mesquite is one of a very few if not the only plant of the many hundreds which have been grown at Pasadena which exhibited maximum growth at these exceedingly high temperatures. Actually, at the Tucson greenhouse mesquite has been shown to survive for extended periods at day temperatures approaching 50° C. Its fair growth even at lower temperatures, together with its previously determined ability to germinate and grow under extreme variations in soil moisture and aeration, clearly demonstrates several of the factors which make possible such aggressive invasion by this noxious plant.

In addition to plants preserved for anatomical studies, the dried plants are being given to Dr. Edwin Kurtz of the University of Arizona, who has expressed an interest in investigating the lipids and waxes of mesquite grown at various temperatures. Findings of these investigations will be included in the 1956 Annual Report.

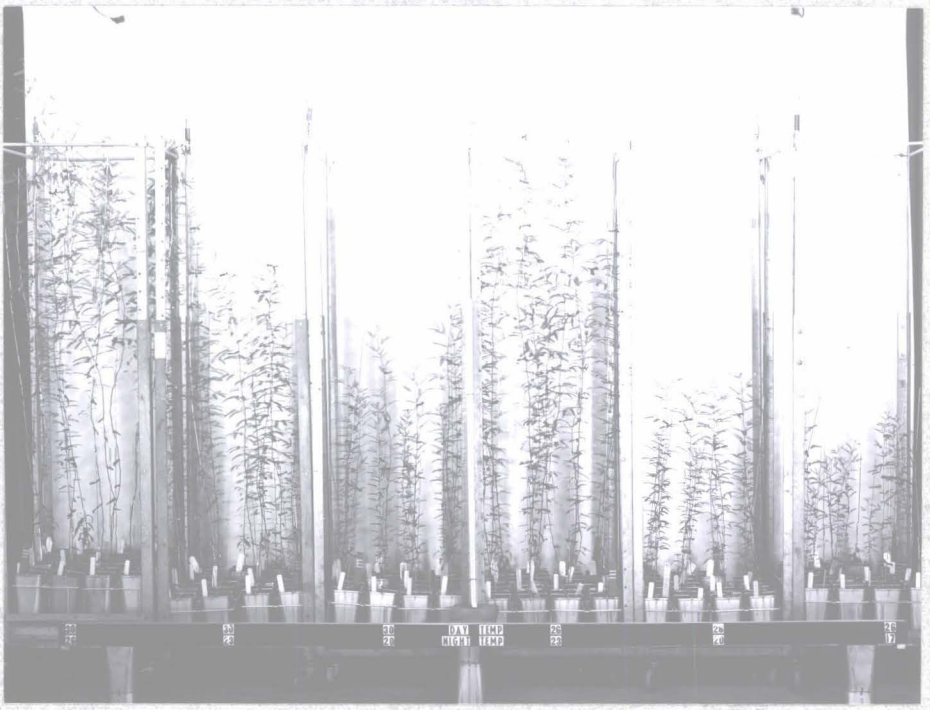

Figure 1. Growth of velvet mesquite plants under six different conditions of day and night temperature, as indicated. Age, 97 days. Entire groups shown.

Figure 2. Similar to figure 1, except selection has been made of the largest and the smallest plant in each temperature group. Note meter stick in center.

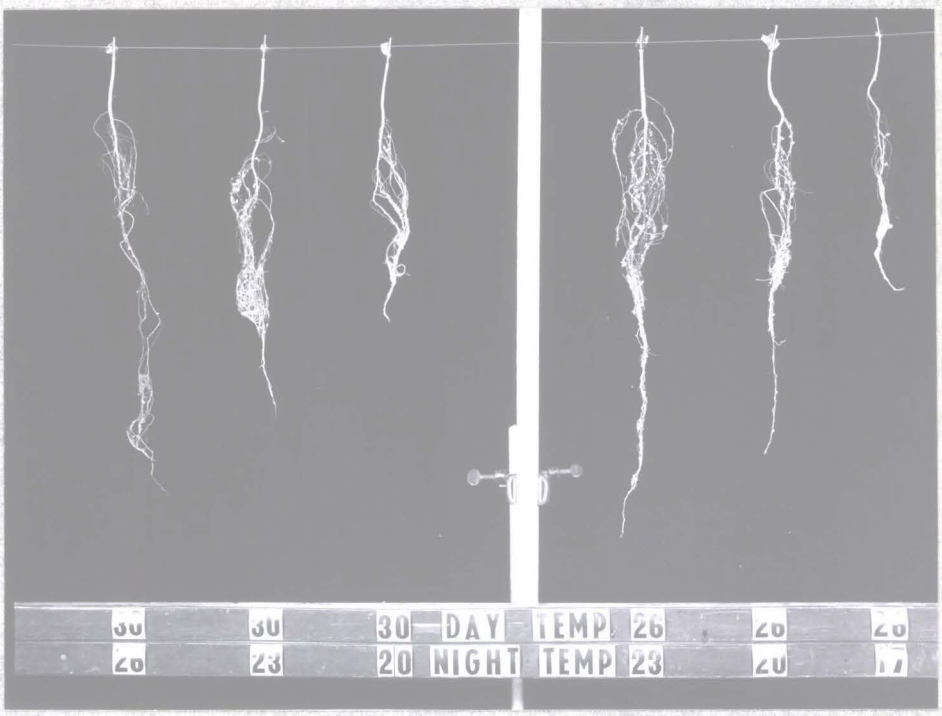

Figure 3. Mesquite roots, the plants of which were grown under temperature condition indicated. Age, 97 days. Relative to plant size, these roots are much smaller than would be found under field conditions.

Preliminary studies on mineral nutrition and the influence of nutrition on herbicidal response.

In the 1954 Annual Report the growth of mesquite seedlings was reported to be slightly better in 50, 25, and 10 percent Hoagland's solution than in 100 percent, when the solution was renewed bi-weekly and adjusted to pH 6.0. To further test the effect of concentration on growth, small seedlings were germinated in distilled water and transplanted to Hoagland's nutrient of 10, 5, and 2 percent concentration, made up in distilled water. Plants cultured under the different conditions all grew to the same size. Those in the 10 and 5 percent solutions were dark green and of vigorous appearance, whereas those in the 2 percent solution were slightly chlorotic in spite of added iron and pH adjustment to 6.0. These experiments indicate that physiological investigations in mesquite, particularly those dealing with individual nutrient elements, are best carried out at low nutrient concentrations where antagonistic and osmotic effects are minimized.

In numerous instances field mesquite has been observed growing in soil of a fairly high salinity. It was considered desirable to have some knowledge of the tolerance of mesquite for the sodium salts, sodium being relatively high in certain of the Southwestern soils, particularly in low rainfall areas where these soluble salts are not excessively leached. A preliminary experiment was set up in which seedlings were grown in complete nutrient solution with the addition of sodium chloride. Five plants were grown at each of four levels of salt concentration, namely 0, 500, 2,000 and 10,000 ppm. At an age of two months, plants grown at the three lower levels had mean heights of 14.0, 13.3 and 17.4 cm. respectively. The highest concentration was lethal to all plants. The group growing at 2,000 ppm. was extremely

variable, one plant being dead and one having a height of 30.8 cm. The latter plant was considerably more vigorous than any of the others - even those grown at lower salt concentrations. The possibility of this plant being a sodium-requiring mutant is tempting, but unlikely. The experiment does demonstrate the relatively high salinity under which mesquite is able to grow well, and suggests the possibility that it may have a sodium requirement in micro-amounts, such as has been shown for sugar beets by Sayre. A more comprehensive experiment would be required to establish such a requirement.

Preliminary experiments had indicated that mesquite seedlings grown in culture solution deficient in magnesium, nitrogen or phosphorous had a different herbicidal sensitivity from plants grown in complete nutrient. A phosphorous (P) deficiency, for example, resulted in an increased sensitivity. Since P is highly variable and often deficient in Southwestern soils, an additional experiment was carried out with this element.

Seeds were germinated in Petri dishes in distilled water, the young seedlings later being selected and transplanted into jars containing 25 percent Hoagland's nutrient. Sixteen plants were grown therein at each of four levels of P, the top level being 8 ppm. - the normal content in 25 percent Hoagland's. The remaining nutrients contained 0.8, 0.08 and 0 ppm. of P respectively. All nutrients were renewed biweekly and adjusted to pH 6.0. At an age of two months, 10 of the 16 plants in each group were treated with 5,000 ppm. of the triethylamine salt of 2,4,5-T carried in a 20 percent aqueous emulsion of nontoxic oil (Sovaspray 100, Socony-Vacuum) with the addition of 0.3 percent emulsifying agent (Multifilm 403).

Application was made by painting the upper surface of the central five leaves. The remaining six plants of each group were not treated but were maintained in the nutrients of varying P content. At time of treatment, only the plants lacking P showed significantly repressed growth and chlorosis of the cotyledons and older leaves. At an age of three months, the mean hight of plants not treated with herbicide was 64.6, 45.5, 38.0 and 9.7 cm. respectively for the 8, 0.8, 0.08 and 0 ppm. concentrations of P.

Total P was determined in the shoots and roots of non-treated plants by a spectrophotometric method. P in the shoots ran 14.2, 6.8, 4.3 and 3.9 mg. per gram dry tissue for the four respective levels of nutrient P. Equivalent values for the root P were 6.4, 3.9, 1.8 and 1.5 mg. per gram dry weight. The significant amounts of P in plants grown in nutrient lacking P were apparently due partially to P originally present in the seed endosperm, and also to the possibility of traces of P being in certain of the nutrient chemicals.

As to the treated plants, contact injury from the herbicide and epinasty of the apical portion of the stem five days after treatment were not significantly different at any of the four levels of P. However, by the third week after herbicide application both the apical portion of the stem and the young leaves were dead in the plants lacking P. These organs showed only moderate injury in plants grown at the other three concentrations of P. By the fourth week, callus formation had appeared on virtually all plants cultured at the three higher levels of P, but in only 1 of the 10 plants lacking P did callus appear. Dieback and percentage kill two and three months respectively after treatment, were maximum in the plants lacking P. The mean dieback, as

a percentage of stem length was 16, 33, 26 and 65 (8 to 0 ppm. content of P respectively), and percentage kill was 10, 0, 20 and 60. None of the non-treated plants of any group died.

It is hoped that a more comprehensive study plan dealing with the effects of P and other nutrient elements can be initiated when time permits. Variation in mineral nutrition may well be one of the yet unexplained causes of erratic field response to herbicides.

Comparative growth and herbicidal response of honey mesquite and velvet mesquite.

The question has often been asked whether honey mesquite (Prosopis juliflora var. glandulosa) has approximately the same herbicidal response as velvet mesquite (P. juliflora var. velutina). Honey mesquite is found in Texas and parts of Oklahoma and New Mexico whereas velvet mesquite is concentrated in the southeastern half of Arizona. Although the two varieties are thus widely distributed geographically, they do grow at approximately the same latitude and consequently receive about the same day lengths. In addition, altitude, soil type and mean temperature are similar in many parts of the respective ranges. Precipitation and humidity are somewhat greater in the areas occupied by honey mesquite. Several of these comparative values at the sites of seed collection in Dickens County, Texas and Pima County, Arizona, are shown in the following table:

	Dickens County, Texas (Near Spur)	Pima County, Arizona (Near Tucson)
Elevation	2274 ft.	2452 ft.
Latitude	33° 28' N.	32° 14' N.
Annual precipitation	21.30 in.	11.16 in.
Mean temperature, January	40.9° F.	49.6° F.
Mean temperature, July	82.0° F.	85.1° F.

Slight differences in herbicidal response that have been noted between the two varieties may simply be due to differences in geographic, edaphic and climatic factors or again may be due to actual differences between the plants themselves.

In order to evaluate the relative growth rate and herbicidal response of the two varieties, both were grown side by side in the greenhouse at Tucson. Thirty seeds of each variety, two years of age, were germinated (after scarifying) and grown in vermiculite in No. 10 cans. Thirty (100 percent) of the honey mesquite seeds germinated, whereas 20 (67 percent) of the velvet mesquite seeds germinated. Growth of the two varieties was very different. At an age of one month the mean height of all honey mesquites which had germinated was 2.4 cm. while the velvet mesquites were 5.7 cm. At this time (December 31, 1954) the velvet variety began to grow considerably faster in height, so that at an age of 3 months the mean heights were 4.0 and 22.2 cm. respectively. The honey mesquite had a low bushy appearance with short internodes, whereas the velvet mesquite invariably had a single stem with longer internodes.

Because of the difference in growth habit it was difficult to give identical herbicidal treatments to both varieties. To minimize this difference, a given percentage of the foliage was treated in each case. Thus, 10 plants of each variety were selected and the leaves making up the central one-third of the foliage were treated on the upper surface with 5,000 ppm. of the triethylamine salt of 2,4,5-T. The herbicide was carried in a 20 percent aqueous emulsion of nontoxic oil with 0.3 percent emulsifying agent. In general, responsive differences of the two varieties were not great. Although contact injury

to the treated leaves was moderate in both cases, 7 of the 10 honey mesquites showed severe damage to the apical non-treated leaves three weeks after treatment. On the other hand, the basal leaves of the honey mesquite showed considerably less chlorosis than those of velvet. By the eighth week there was no dieback in any of the honey mesquite stems, but 40 percent of the velvet mesquite plants had dieback for approximately three-fourths of the stem length. Three months after treatment only one of the 20 plants was dead, it being a honey mesquite.

The considerably slower winter growth of honey mesquite as compared to velvet is difficult to explain. It is unlikely that temperature was much of a limiting factor because the greenhouse is heated during the winter to approximately 30° C., day, and 22° C., night. With the lengthening days of spring growth rate of the non-treated honey mesquite controls began to pick up, but even by June they were still considerably shorter than velvet mesquite. It appears likely that day length may be an important factor governing the growth of mesquite, particularly the honey variety. The possibility exists that the two varieties have a slightly different critical day length for growth, and that in the case of honey mesquite the days were simply not long enough to reach the critical value during mid-winter.

Although there were significant differences in specific herbicidal responses in the two varieties, the overall response was not sufficiently different to allow the statement that one variety is more sensitive or is more readily killed by 2,4,5-T than the other.

Effect of the ratio of oil to water in herbicidal carriers.

Although past aerial sprays have usually indicated an emulsion of diesel oil in water to be slightly more efficient than water only as a carrier for 2,4,5-T, hand sprays on field mesquite have shown that water alone is an excellent carrier. In a number of instances, very good results have been achieved by aerial application, utilizing a water carrier, but not consistently. If a water carrier or a carrier having a very low percentage of oil could be made to consistently give as good results as a 1:4 or 1:7 oil-water emulsion in aerial application, a considerable saving could be realized as well as greater convenience in formulating the herbicide mixture in the field and keeping it properly emulsified. Laboratory experiments have shown that in order to keep an oil-water emulsion stable for an hour or more without continuous agitation, an emulsifying agent must often be added at a concentration that is supraoptimal for maximum herbicidal effect. An emulsion, for example, of an ester of 2,4,5-T in water only is generally quite stable even with the low concentration of emulsifying agent provided in most commercial herbicide formulations and usually works satisfactorily in aircraft tanks not equipped with agitating devises. This is not always the case when an oil-water emulsion is employed.

To evaluate the effect of ratio of oil to water in the carrier, an experiment was designed utilizing varying ratios of nontoxic oil (S/V Sovaspray 100) to water. Thus, carriers were made up with the following volume ratios of oil to water: 100 percent oil, 1:1, 1:4, 1:9, 1:29, 100 percent water. The herbicide consisted of 5,000 ppm.

of the triethylamine salt of 2,4,5-T in all carriers containing water. In the straight oil carrier an oil soluble amine (ACP-L-965) was used at an equivalent acid concentration. An anionic emulsifying agent (Multifilm 403) was added at 0.3 percent concentration. Only the upper surface of three centrally located leaves of each plant was painted with a small camel's hair brush.

Initial response to the herbicide was remarkably similar in all six treatments. The spotted bleaching which was apparent on the treated leaves after the fifth day was slightly less intense in the case of the water-only carrier than with the others. Approximately 15 percent of the leaf area was bleached as compared to 20 to 40 percent with the other carriers. Formative effect to the apical bud and young non-treated leaves was moderately severe and approximately equivalent in all treatments. Effect on basal leaves, mainly chlorosis, was mild, but again was similar in all treatments. Callus formation was quite prevalent on the stems of plants in all treatments except those receiving the straight oil carrier. No callus was evident in the latter case.

Two months after treatment, differences became more apparent. Dieback of the stem at that time, as a percentage of total stem length, was greatest in the plants receiving the water only carrier. The mean percentage values for each of the 6 treatments, starting with 100 percent oil were: 20, 45, 48, 33, 46, 68. Percentage kill at the end of the third month was also greatest with the water carrier. Of the four plants out of 60 which ultimately died, all were from the latter group.

Had diesel oil been used in this investigation rather than non-toxic oil, the differences between the various emulsions and the water only carrier would probably have been greater, judging from the results

of earlier experiments in which both types of oil were used. This experiment clearly demonstrates that for greenhouse seedlings, where penetration and translocation distances are at a minimum, the most effective carrier is water. In addition, the good kills often achieved in the field by the use of a water carrier (though not as consistently as with an oil-water emulsion) suggest that the water carrier should not be completely excluded in future research programs on mesquite, as has been the trend the last few years.

The effect of pH upon herbicidal efficiency of the free acid of 2,4,5-T.

Several investigators have demonstrated an enhanced absorption and general herbicidal effect by increasing the acidity of the herbicidal mixture before application. Since such experiments had not been attempted on velvet mesquite, several preliminary tests were designed for greenhouse-grown seedlings. Granules of the free acid of 2,4,5-T at 5,000 ppm. were used as the herbicide in all cases, as this material had previously given excellent results in greenhouse evaluations. Since the above concentration of 2,4,5-T is nearly 20 times its solubility in water, the herbicide was applied essentially as a suspension. The formulation used contained a 90 percent Bentonite filler. In all cases it was made up in a 20 percent emulsion of nontoxic oil in water with the addition of 0.3 percent of an anionic emulsifying agent. Four pH levels were used, namely 2.0, 4.0, 6.0, and 8.0, the adjustment being made with trichloroacetic acid (TCA) or ammonium hydroxide. Although any herbicidal effects of the TCA would be superimposed upon the pH effects at the lower values, personal communication with one investigator had indicated beneficial effects when acidifying with TCA, and it was consequently used in the first investigations.

Forty selected seedlings, 15 days of age were utilized for the experiment - 10 for each pH level. At this age the plants had the cotyledons out as well as 3 foliage leaves. The herbicide was painted on the upper surface of the cotyledons only. Initial contact injury five days after treatment held no linear relationship to pH value. At the two extreme values of 2.0 and 8.0, no visible injury was discernible on this date, whereas considerable shrivelling was apparent

on the cotyledons receiving the herbicides of intermediate pH values. Also on this date, the pH 2.0 plants showed moderate epinasty in the apical portion of the stem, whereas those in the other groups had only very slight epinasty. Shortly after this observation a rodent destroyed many of the plants, and consequently the remainder of the observations were made only on four uninjured plants in each group. Three weeks after treatment, plants receiving the pH 2.0 herbicide were severely chlorotic in the apical portion of the stem and the young leaves. The other groups showed only slight chlorosis in this region. In addition, stem callus was considerably more profuse in the pH 2.0 group. By the eighth week this group also had a mean stem dieback of 20 percent of the plant height, whereas plants receiving the other treatments had no dieback. The mean height of the pH 2.0 plants at this time was about half that of the other three groups; the plants essentially stopped growing within a day or two of herbicidal application. By the third month, two plants in the pH 2.0 group had died, and one in the pH 8.0. Perhaps significant is the fact that these two groups are the same ones which originally received <u>least</u> initial contact injury on the treated cotyledons. The experiment points out the fact that lowering the pH value of the herbicidal mixture to 2.0 under the conditions specified is definitely beneficial. Of the various responses observed, no significant differences were found among plants in the three higher pH groups.

In order to determine whether the above described responses would be similar when the herbicide was applied to centrally located leaves rather than cotyledons, another experiment was run. The same herbidde and carrier combination was used and this time was adjusted to three

pH levels, 2.3, 4.0 and 6.0. Twelve plants were used for each treatment, and the herbicide was painted on the upper surface of the central five leaves of each plant. Initial chlorosis of the treated leaves five days after application, was moderate to severe in the pH 2.3 group, whereas treated leaves of the other groups were even more severely chlorotic and bleached. Mean initial epinasty of the apical stem was slightly greater for the pH level of 2.3 than for the other two. Although later callus formation adjacent to nodes of the treated leaves was more profuse in the pH 2.3 group, formative effect and chlorosis of the apical bud and young leaves was most severe in the pH 4.0 group. This observation corroborates earlier work in which it was noted that severe callus formation often resulted in decreased herbicidal responses beyond the callus. Dieback was not greatly different among the three groups, and as to percentage kill after the third month, only 2 plants were dead in each of the pH 2.3 and 4.0 groups. This experiment suggests that herbicidal responses resulting from application to the leaves are less dependent upon pH level than when application is made to the cotyledons.

To help clarify some of the relatively small responsive differences occurring when leaves as compared to cotyledons are treated at several pH levels, one further experiment was installed. Ninety plants were selected which averaged about 7 cm. in height and had three leaves. They were divided into two groups, one of which was treated on the upper surface of the cotyledons while the other was treated on the upper surface of the single primary leaf, immediately above the cotyledon. The two organs had approximately an equivalent surface area. The previously mentioned formulation of 2,4,5-T was used, and was adjusted to each of

three pH levels, namely 2.0, 5.0 and 8.0. Phosphoric acid rather than trichloroacetic was used for pH adjustment to the lower levels, thus avoiding possible herbicidal effects resulting from the latter acid. Herbicides of each pH level were applied to 15 plants in each of the cotyledon-treated and leaf-treated groups.

The previously described responses were in many cases not significantly different either among individual pH values or between the general cotyledon-treated and leaf-treated groups. The lack of contact injury to cotyledons when the herbicide mixture was acidified to pH 2.0 with TCA did not follow when the pH was adjusted with phosphoric acid. Apical epinasty was considerably greater in the leaf-treated group as a whole, and within this group was significantly greater at the pH 2.0 and 5.0 levels than at 8.0. Actual chlorosis and formative effects to the apical bud and young leaves three weeks after treatment were equivalent in the two major groups, but within each group the greatest effects were noted at pH 5.0. Callus was by far the most extensive in the leaf-treated plants, where it generally formed only on the stem between the cotyledonary node and the node of the primary treated leaf. When the cotyledons were treated, what little callus formed was generally distributed equally along the entire stem, with the exception of the apical one or two internodes. None of the 90 plants was killed as a result of the treatments.

It is of interest that many of the cotyledons in the leaf-treated group were still green three weeks after application of the herbicide. Thus, of the 30 cotyledons (15 plants) in each pH group, 18, 23, and 11 respectively, were still green in each of the pH groups of 2.0, 5.0 and 8.0. All treated cotyledons were of course dead. Strangely

enough, all cotyledons on 45 non-treated control plants of the same age were dead at this time. Apparently little or none of the herbicide that was absorbed and translocated from the single primary leaf moved into the cotyledons. Presumably, metabolism in the remainder of the plant was sufficiently repressed by the herbicide absorbed through one leaf to result in decreased utilization of the stored foods in the cotyledons, with the result that the latter survived considerably longer than in non-treated plants.

In summary, the experiments on pH suggest a somewhat enhanced effect at the lower values under certain conditions. Differences in certain herbicidal responses resulting when the leaves are treated as compared to the cotyledons suggest possible differences in chemical or physical structure of these organs - something which would of course be expected. It is known that cotyledons are more sensitive than leaves to air pollutants consisting principally of organic peroxides, ozone and other highly-oxidizing substances, whereas leaves are more sensitive than cotyledons to air-borne toxicants such as sulfur dioxide. Such variable responses also indicate differences that likely exist between these two organs.

The influence of emulsifier concentration upon herbicidal effectiveness on field grown velvet mesquite, accompanied by different forms of 2,4,5-T and different carriers.

Preliminary greenhouse experiments with 2,4,5-T had indicated the relative importance of emulsifier type and concentration in the final herbicidal mixture, and consequently a field study was initiated with this variable in mind. The study was installed during September of 1954 and observed after one month. The statistical design and other details of the experiment are described in the 1954 Annual Report, along with results of the first observation. A second observation was made after one year, during September of 1955. This observation gives a more accurate prediction of actual plant kill, but final results will not be known until the summer of 1956.

Briefly, the treatment utilized two carriers, one a 20 percent emulsion of nontoxic oil (S/V Sovaspray 100) in water, and the other a similar emulsion of diesel oil in water. Two individual formulations of 2,4,5-T (without other additives) were used with each carrier, namely 12,000 ppm. of the triethylamine salt and a similar concentration of the butoxyethanol ester. Thus, four basic classes were used. Each class was further subdivided by emulsifier concentration. Five concentrations of an anionic emulsifier (Multifilm 403) were employed in each case, as follows: 0.0, 0.1, 0.4, 0.8 and 1.2 percent by volume, of the total emulsion. A total of 400 trees was hand sprayed, 20 with each of the 20 possible herbicide combinations.

It has often been considered that initial defoliation and final

kill of mesquite generally are not correlated. However, reference to table 3, which shows the mean percentage of foliage remaining on the trees both one month and one year after treatment, indicates a very definite relationship between the two observation dates. Of greatest interest is the fact that the ester formulation of 2,4,5-T in a diesel oil emulsion has given best results. Within this treatment, there is a positive correlation of emulsifier concentration and effectiveness of the herbicide, as evidenced by the amount of foliage remaining on the trees. The concentration of 1.2 percent gave by far the best results of all 20 treatments. This fact suggests that in future experiments an even higher emulsifier concentration should be used. Considering the 1-year observation, none of the 3 other basic treatments had significant intrinsic correlations of emulsifier concentration with foliage remaining on the tree - or probable percentage kill. Relatively poor herbicidal effect was obtained in the complete absence of emulsifier, with the exception of the ester-nontoxic oil emulsion combination. Here, only at the highest emulsifier concentration was a significant benefit obtained.

An analysis of variance of the one-year observations, attended by 396 degrees of freedom, indicated highly significant differences between carriers, emulsifier concentrations, and the interactions of carrier by formulation and emulsifier concentration by formulation. Differences between herbicide formulation alone and other combinations of the three variables were nonsignificant. Means of the grouped treatments are shown in table 3.

The optimum effect achieved by the highest emulsifier concentration of 1.2 percent was an unexpected occurrence, since this is a

Table 3. Percentage of foliage remaining green on velvet mesquite trees sprayed with 12,000 ppm. 2,4,5-T in the formulation, carrier, and emulsifier concentration indicated. Summary of 400 trees, one month and one year after treatment. Mean and standard error of the mean shown.

Emulsifier concentration (Percent)	Triethylamine salt of 2,4,5-T				Butoxyethanol ester of 2,4,5-T				Mean of combined herbicide formulations and carriers.	
	Diesel oil emulsion		Nontoxic oil emulsion		Diesel oil emulsion		Nontoxic oil emulsion			
	1 month	1 year	1 month	1 year	1 month	1 year	1 month	1 year	1 month	1 year
0.0	19 ± 4	49 ± 5	33 ± 7	54 ± 4	40 ± 8	46 ± 8	50 ± 9	56 ± 8	35 ± 4	51 ± 3
0.1	18 ± 5	40 ± 5	16 ± 4	41 ± 7	26 ± 4	46 ± 7	55 ± 8	65 ± 8	28 ± 3	48 ± 3
0.4	16 ± 3	42 ± 5	10 ± 2	40 ± 5	10 ± 3	28 ± 6	54 ± 7	60 ± 6	22 ± 3	43 ± 3
0.8	9 ± 2	40 ± 4	21 ± 4	50 ± 5	14 ± 4	32 ± 6	60 ± 6	67 ± 5	25 ± 3	47 ± 3
1.2	12 ± 2	46 ± 6	9 ± 2	46 ± 6	5 ± 1	16 ± 2	30 ± 5	41 ± 6	14 ± 2	37 ± 3
Mean, all emulsifier concentrations	14 ± 2	43 ± 2	18 ± 2	46 ± 2	19 ± 2	34 ± 3	49 ± 3	58 ± 3	25 ± 2	45 ± 2

higher concentration than commonly used. It is also considerably higher than generally results when most commercial herbicides formulated with an emulsifier are diluted up to recommended volume. It should be remembered that herbicidal application was made during September, which is approximately three to four months later than the mean period of optimum sensitivity. Whether or not the same treatment would have given optimum results during May, for example, cannot be told from this experiment. The possibility exists that cuticular and cell wall structure of the leaves changes considerably during the year, and consequently an emulsifier concentration which enables maximum penetration of a given herbicide at one time of the year may not be an optimum concentration at another time of year. Experiments to the present time have not clearly defined whether or not the optimum period of plant susceptibility holds for all formulations of 2,4,5-T as well as for different carriers. Certainly the effect of emulsifier type and concentration has not been considered. It is hoped that currently installed studies and others contemplated for the near future will help clarify some of these problems.

Absorption and translocation of several relatively new herbicides in velvet mesquite seedlings.

Screening tests of certain new herbicides were continued during the year, utilizing the same technique described in the 1954 Annual Report. Experimental plants were used at an age of two months, and were all progeny of a single parent to minimize possible genetic variation. The centrally located three or four leaves of each plant, amounting to approximately one-third of the foliage, were treated by painting with a small camel's hair brush. In all tests carried out during 1955, the herbicide was applied at 5,000 ppm. acid equivalent and the carrier consisted of a 20 percent emulsion of nontoxic oil (S/V Sovaspray 100) in water, with the addition of 0.2 or 0.3 percent of an emulsifying agent (Multifilm 403). In earlier experiments this combination had resulted in optimum absorption and/or translocation. In addition the oil carrier, being completely non-phytotoxic by visual determination, allows a more accurate appraisal of the contact effects of the herbicide itself.

In the first experiment, 10 plants were treated in the above described manner with each of the following herbicides: granules of 2,4,5-T (free acid with 90 percent Bentonite filler); triethylamine salt of 2,4,5-T (ACP-L-282); 2(2,4,5-trichlorophenoxy) propionic acid, mixed ethanol and isoproponol amines (Silvex, M-213); 2(2,4,5-trichlorophenoxy) propionic acid, propylene and polypropyleneglycolbutylether esters (Silvex, M-235); 2(2,4-dichlorophenoxy) propionic acid, mixed ethanol and isoproponol amines (A-1106); 2(2,4-dichlorophenoxy) propionic acid, mono, di and tripropyleneglycolbutylether esters (S-1280).

The prime objective of this experiment was to compare the relative effects of the propionic acids (2,4-DP and 2,4,5-TP) and in turn to compare such effects with those resulting from the free acid and triethylamine formulations of 2,4,5-T, which have thus far given optimum results in greenhouse evaluations. Responses at various intervals after treatment are described in table 4. In considering the overall efficacy of the different herbicides, it is probably well to omit the contact effect on treated leaves and intensity of callus formation, since these responses are not necessarily desirable when working for maximum translocation and plant kill. The implication of these responses was discussed in the 1954 Annual Report. By summation of the other responses listed it is evident that the two formulations of 2,4,5-T were the most effective of all herbicides tested, under the conditions of this experiment. Of the four propionic acids, the two amine formulations gave least contact injury, but slightly better overall responses than the corresponding esters. Considering the amines, formative effect to the apical leaves three weeks after treatment was slightly greater with 2,4,5-TP than with 2,4-DP, but the later and probably more important responses of dieback and plant kill were greater with the dichloro form. Although not great, the difference is rather surprising in view of the fact that 2,4,5-T has generally been found superior to 2,4-D in most experimental work on mesquite.

A second experiment of similar design was run utilizing two different commercial formulations of the butoxyethanol ester of 2,4,5-T (ACP-L-329 and ACP-L-533) and the butoxyethanol ester of 2(2,4-dicholophenoxy) propionic acid (ACP-L-685). Fourteen plants of the same age as in the previous experiment were used for each treatment, as well as

the same carrier, etc.

The responses of plants in this experiment (taken at the same intervals shown in table 4) are not tabulated since they were all very similar, and practically identical to the response of the last listed herbicide in table 4 (2,4-DP ester). There was no dieback or kill of any plants in the three groups. Growth repression was slightly less in plants receiving the 2,4-DP formulation as compared to those receiving the 2,4,5-T formulations, as plants in the former group were somewhat larger than the others, three months after treatment. They were far smaller than the non-treated controls however.

In summary, the free acid or amine formulation of 2,4,5-T appears to give considerably more effective results than similar formulations of 2,4,5-TP or 2,4-DP in greenhouse evaluations. Results of field tests involving the propionic acids are still not final. They may prove relatively more effective in the field. As to the ester formulations, greenhouse experiments suggest nonsignificant responsive differences between 2,4,5-T and the propionic acids.

Table 4. Certain responses of mesquite seedlings resulting from application of 5000 ppm. acid equivalent of the herbicide indicated to centrally located leaves only. Carrier 1:4 emulsion of nontoxic oil in water with 0.3 percent emulsifying agent.

Herbicide	Response after six days		Formative effect on young leaves after three weeks	Response after five weeks		Mean dieback of stem as percentage of total stem length after nine weeks	Number of plants dead out of 10 by 12th week
	Contact effect on treated leaves	Degree of apical epinasty, and percentage of plants so affected		Intensity of callus on stem, and percentage of plants so affected	Effect of basal leaves		
2,4,5-T, acid granules	Very slightly chlorotic, entire leaf	Moderate, 100	Severely chlorotic or shriveled, 40% of plants	Moderate, 50	Moderately chlorotic	72	8
2,4,5-T, triethylamine	Small bleached spots covering 5% of leaf area	Moderate, 100	Very severely chlorotic or shriveled, 70 % of plants	Moderate, 60	Moderately chlorotic	44	3
2(2,4,5-Trichlorophenoxy) propionic acid, amine	Small bleached spots covering 10% of leaf area	None	Severely chlorotic or shriveled, 20% of plants	Slight, 70	No effect	3	0
2(2,4,5-Trichlorophenoxy) propionic acid, ester	Severely bleached, entire or outer half of leaflets	None	No effect	Moderate, 30	No effect	0	0
2(2,4-Dichlorophenoxy) propionic acid, amine	Bleached spots covering 10% of leaf area	Slight, 10	No effect	Very slight, 10	Slightly chlorotic	20	2
2(2,4-Dichlorophenoxy) propionic acid, ester	Severely bleached, entire leaf	None	No effect	None	No effect	0	0

Herbicidal effects of 2,4,5-T, amino triazole and trichlorobenzoic acid, alone and in various combinations.

Preliminary experiments with 3-amino-1,2,4-triazole (AT) on velvet mesquite seedlings, as described in the 1954 Annual Report, have shown that it produces marked physiological responses but does not have the killing power of 2,4,5-T. Its possible synergistic effect when used as an additive to the latter herbicide has been suggested by several investigators. To test such an effect of AT, as well as 2,3,6-trichlorobenzoic acid (TCB) and sodium 2,3,6-trichlorobenzoate (NaTCB), these compounds were used alone and in various combinations on velvet mesquite seedlings. Since AT had previously been tested alone, it was used only as an additive in the present experiment.

All herbicides were applied at a concentration of 5,000 ppm. In addition, NaTCB was applied at 10,000 and 20,000 ppm. Where two herbicides were combined, each was used at 5,000 ppm. In every case the carrier consisted of a 20 percent emulsion of nontoxic oil in water with the addition of 0.3 percent of an anionic emulsifying agent. TCB , being oil soluble, was dissolved in the oil phase of the carrier before emulsification. The other formulations were first dissolved in the water phase.

One hundred and twenty vigorously growing, five-week-old plants were selected and divided into 12 groups of 10 plants each. One group was maintained as a control, and the others were treated with the herbicide mixtures indicated in table 5, by means of painting the mixture on the upper surface of the central four leaves of each plant. The plants had an average of 12 leaves at this stage of growth. Since all recorded

Table 5. Various responses of velvet mesquite seedlings to the following herbicides, alone and in several combinations: 2,4,5-T, triethylamine salt (2,4,5-T); 3-amino-1,2,4-triazole (AT); 2,3,6-trichlorobenzoic acid (TCB) and sodium-2,3,6-trichlorobenzoate (NaTCB). All herbicides carried in a 20 percent emulsion of nontoxic oil in water. Ten plants per treatment.

Herbicides or combinations (See above) Concentrations 5000 ppm. a.e. except as indicated	Response after six days (1 = very slight, 5 = very severe, etc)		Condition of young leaves after three weeks	Response after five weeks		Response after nine weeks	
	Severity of spotted bleaching on treated leaves	Degree of apical epinasty		Intentsity of callus on stem, and percentage of plants affected (1 = v. slight, etc.)	Number of plants sprouting, and those with sprouts developing into "micro leaves" (M)	Total number of sprouts per 10 plants	Mean dieback of stem as percentage of total stem length
2,4,5-T	4	4	Dead	4 - 60	2	7	40
TCB	4	4	Very slightly chlorotic	None	3	25	1
NaTCB	3	3	Green	"	6,M	27	0
NaTCB (10,000 ppm.)	4	2-3	"	"	7,M	28	0
NaTCB (20,000 ppm.)	4	3-4	"	"	8,M	24	0
2,4,5-T + AT	2	3-4	Slightly chlorotic	5 - 70	7	20	11
2,4,5-T + TCB	5	3	Variable mostly green	3 - 50	7	18	17
2,4,5-T + NaTCB	4	4	Very slightly chlorotic	4 - 80	6	18	7
AT + TCB	2	3	Green	None	9, semi M	21	0
AT + NaTCB	4	3	"	"	10, semi M	37	0
TCB + NaTCB	5	3-4	"	"	10, semi M	41	0

responses in the control plants were completely negative, they are not shown in the table.

Contact injury to the treated leaves, observed six days after herbicidal application, was moderate to severe in all cases, with two exceptions. The addition of AT to either 2,4,5-T or TCB significantly lessened contact injury resulting from either of the latter herbicides alone. (Contact injury resulting from AT alone would be insignificant at this date.) On the other hand, the addition of AT to NaTCB increased contact injury over that caused by NaTCB alone. Treated leaves on plants receiving the three herbicide combinations containing AT never bleached completely as was the case on plants receiving all other herbicides, but turned first to a light green and ultimately to a light gray.

The combination of TCB with either 2,4,5-T or NaTCB caused very severe contact injury. It is of interest that in each of these combinations one oil soluble and one water soluble herbicide is involved, so that each of the two phases of the emulsion contains an herbicide. Of the three concentrations of NaTCB used, 5,000, 10,000 and 20,000 ppm., all gave virtually the same overall response.

Although there was a considerable amount of apical epinasty six days after treatment with all herbicides and combinations (with probably no significant differences), a rather unusual physiological response occurred in the plants receiving the TCB and NaTCB. The terminal portions of the rachises of the young pinnately compound non-treated leaves showed considerable epinasty. The resulting appearance (figure 4) was quite different from effects caused by previously used herbicides, which generally result in some degree of chlorosis to the young leaves but epinasty only in the stem or basal portion of the petioles.

Figure 4. Epinasty of both the apical portion of the stem and the rachis of a young non-treated leaf. Lower leaves treated with 5,000 ppm. trichlorobenzoic acid in a 20 percent emulsion of non-toxic oil in water, eight days prior to photograph.

Chlorosis or marked injury to the young leaves by the third week and stem callus formation appeared only on plants treated with 2,4,5-T, alone or in combination with other herbicides. A very slight chlorosis was noted in the plants treated with TCB , however.

Considering the combined responses of injury to the non-treated leaves and dieback of the stem, 2,4,5-T alone gave outstanding results. When combined with any of the other herbicides its efficiency, as measured by these two important responses, was essentially cut to half or less. Sprouting was also minimum on plants treated with 2,4,5-T only. Leaves which developed from sprouts of plants treated with NaTCB or TCB alone or in certain combinations with other herbicides (see table 5) were markedly stunted and were termed "micro leaves". Although seemingly physiologically mature, many of these leaves never reached a size greater than about one-fourth that of normal leaves (figure 5).

Under the conditions of this experiment, it appears that the herbicides other than 2,4,5-T when used alone are considerably less effective than 2,4,5-T, and when used in conjunction with the latter herbicide are only antagonistic toward its action.

Figure 5. Development of "micro leaves" from sprouts of plants treated 36 days previously with 5,000 ppm. sodium trichlorobenzoate. Leaves of this type never reach normal size.